

Biomanufacturing Module 3

Lesson 1 – Bacterial Lysis

Lesson objectives:

Students will understand:

- How to perform a bacterial lysis.

Essential Question

- Why do you need to lyse the RFP+ (or GFP+) bacteria?

Materials:

- Intro to Downstream Process - Protein Purification slide deck
- Bacterial Lysis Protocol
- p1000 micropipette and tips (1/team)
- 1.5 mL microfuge tubes (2/team)
- Microcentrifuge (1/class)
- p200 micropipette and tips (1/team)
- Elution buffer (TE) (500ul/team)
- Lysis Buffer (BPER) (500ul/team)
- 10% bleach solution in a beaker - sitting in the sink or inside another container (1/team)
- Downstream Process Batch Record Document

What Students Will Do

- Listen to/watch the Intro to Downstream Process - Protein Purification slide deck
- Mix their harvested RFP+ or GFP+ bacteria.
- Follow the Bacterial Lysis protocol
- Each team fills out the appropriate parts of their Downstream Process Batch Record

Teacher Preparation

- Prior to class make copies of
 - Bacterial Lysis Protocol (one per team)
 - Downstream Process Batch Record Document (one per team)
- Prior to class prepare 500uL aliquots of Elution Buffer (TE) (one per team).
- Prior to class prepare 500uL aliquots of Lysis Buffer (BPER) (one per team).
- Prior to class prepare beakers half full of 10% bleach solution (one per team). Put these in the sink or inside of another container - to contain spills
- Provide each team
 - One p1000 micropipette and tips
 - One p200 micropipette and tips
 - Two 1.5mL microfuge tubes
 - Sharpie marker for labeling tubes
 - One 500uL aliquot of Elution buffer (TE)
 - One 500uL aliquot of Lysis buffer (BPER)
 - Spray bottle of 70% ethanol
 - Paper towels

- Team file folders

Organizer

Time	Activity	Materials
15 minutes	Present the Intro to Downstream Process - Protein Purification slide deck	Slide deck
5 minutes	Members of all teams put on PPE	Lab coats, gloves, safety goggles
5 minutes	Teams sanitize and prepare their bench space	70% ethanol, paper towels, micropipettes, tips, harvested bacteria
15 minutes	The Downstream Process Technicians from each team carry out the bacterial lysis protocol.	Bacterial Lysis Protocol, 1.5mL microfuge tubes, tube rack, micropipettes, tips, Elution buffer, Lysis buffer
5 minutes	Teams label their bacterial lysate tubes and place them in a tube rack to incubate at room temperature overnight. Tubes containing harvested bacteria get stored in the refrigerator.	Sharpie markers Tube rack
10 minutes	Teams fill out the appropriate portions of their Downstream Process Batch Record Document and file it	Upstream Process Batch Record Document, Team File Folder

Procedure

Introduction to Protein Production in Bacteria

1. Present the Introduction to Downstream Process - Protein Purification slide deck.

Preparation of the bacterial lysate

2. Members of each team put on PPE
3. Each team sanitizes and organizes their bench space
4. The Downstream Process Technicians from each team follow the Bacterial Lysate Protocol to create a bacterial lysate.
5. Each team puts their tubes containing the bacterial lysate in a tube rack to incubate at room temperature overnight.
6. Each team fills out the appropriate sections of the Downstream Process Batch Record and files it in their team file.

